Red cell membrane disorders – challenges in diagnosis

NARAZAH MOHD YUSOFF
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Red cell membrane disorders

- Inherited diseases
  - Mutations in various membrane or skeletal proteins
  - Decreased red cell deformability (rigidity), reduced life span and premature removal of RBC
  - Characterized by marked clinical and laboratory heterogeneity

Important group of inherited haemolytic anaemias

Significant genetic heterogeneity
Anton van Leeuwenhoek
Father of Microscopy and Microbiology (1675)

Anton van Leeuwenhoek

- Created the first known microscope
  - Using the tiny lenses
  - Magnify 300X (times)
- Discovered a “microscopic world”
  - Red blood cells
  - Pond water “beasties”
  - Muscle fibres

- “when he (patient) was greatly disordered, the globules of his blood appeared rigid, but grew softer and more pliable as his health returned: whence he infers that in a healthy body they (RBC) should be soft and flexible”
The human RBC

✓ discoid shape
✓ ability to undergo extensive passive deformation during repeated passage through the narrow capillaries of the microvasculature

NB: marked deformation the cell undergoes during its passage through the narrow endothelial slit separating the cord from the sinus.

Extensive studies biochemical, biophysical, molecular & biological approaches -> molecular insights into structural basis for normal RBC membrane function and for altered function

Contributed to the improved understanding of their pathophysiology & differences in the severity of clinical manifestations
Simplified cross-section of the erythrocyte membrane.

Immacolata Andolfo et al. Haematologica 2016;101:1284-1294

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Concepts regarding the structural basis for normal RBC membrane material properties

• unfolding and refolding of spectrin repeats accounts for the remarkable elasticity

• Vertical linkages between bilayer and membrane skeleton

• Lateral linkages between spectrin dimers and between spectrin-actin-protein 4.1R

• hinderance of these unfolding results in increased membrane rigidity

• play a critical role in maintaining membrane cohesion

• dominant regulators of membrane mechanical stability
Defects

Defects in proteins involved in linking the lipid bilayer to membrane skeleton (vertical linkages) -> loss in membrane cohesion -> surface area loss (hereditary spherocytosis - HS)

Defects in proteins involved in lateral linkages of the spectrin-based skeleton -> decreased mechanical stability, membrane fragmentation (hereditary elliptocytosis - HE)

Severity is primarily dependent on the extent of membrane surface area loss
Classification, diagnostic criteria of RBC membrane defect-related anemias

- Two main sub groups:
  (a) structural defects
  (HS, HE, hereditary pyropoikilocytosis (HPP), Southeast Asian ovalocytosis (SAO))
  (b) altered permeability of the RBC membrane
  (dehydrated hereditary stomatocytosis (DHS), overhydrated hereditary stomatocytosis (OHS), familial pseudohyperkalemia (FP), and cryohydrocytosis (CHC))
**Classification of erythrocyte membrane disorders by OMIM database (characterised mutations)**

<table>
<thead>
<tr>
<th>Disease symbol</th>
<th>Phenotype</th>
<th>Phenotype MIM number</th>
<th>Gene location</th>
<th>Protein name</th>
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<tr>
<td>HS1</td>
<td>Hereditary spherocytosis type 1</td>
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<td>DHISS1</td>
<td>Dehydrated hereditary stomatocytosis with or without pseudoherpakeriaemia and/or perinatal edema</td>
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<td>PIEZO1-type mechanosensitive ion channel component 1</td>
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| Protein name reported in Uniprot database. AD: Autosomal dominant; AR: Autosomal recessive; ATP: Adenosine triphosphate; Rh: Rhesus; OMIM: Online Mendelian Inheritance in Man; |
hereditary spherocytosis

Defects in proteins involved in linking the lipid bilayer to membrane skeleton (vertical linkages) -> loss in membrane cohesion -> surface area loss
Defects in proteins involved in lateral linkages of the spectrin-based skeleton -> decreased mechanical stability, membrane fragmentation
RBC membrane transport defects

• RBC also play important role in regulating cell volume haemostasis
• Requirement for maintenance of normal RBC volume
• Loss of ability – to regulate its volume, reduce life span of RBC
• Stomatocytoses
  • dehydrated hereditary stomatocytosis (DHS), overhydrated hereditary stomatocytosis (OHS), familial pseudohyperkalemia (FP), and cryohydrocytosis (CHC)
Southeast Asian Ovalocytosis (SAO)

- hereditary RBC membrane defect characterized by the presence of oval-shaped erythrocytes and with most patients being asymptomatic or occasionally manifesting with mild symptoms such as pallor, jaundice, anemia and gallstones.

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**ORPHA:98868**

- **Synonym(s):** Hereditary ovalocytosis, Melanesian elliptocytosis, Melanesian ovalocytosis, SAO
- **Stomatocytic elliptocytosis**
- **Prevalence:** <1/1,000,000
- **Inheritance:** Autosomal dominant
- **Age of onset:** All ages
- **ICD-10:** D581
- **OMIM:** 166900
- **UMLS:** -
- **MeSH:** -
- **GARD:** -
- **MedDRA:** -
SAO is common in Southeast Asian and Western Pacific countries (i.e. Thailand, Malaysia, Indonesia, Philippines and Papua New Guinea).

SAO is very common in malaria-endemic areas (prevalence 1/20-1/4) but in Europe it is very rare.

The prevalence of SAO in Malays was reported at 4.0% in Kelantan (Yusoff et al., 2003) and 4.4% in Kepala Batas, Pulau Pinang (Raman et al., 2014).
Newborns with SAO might be symptomatic with haemolysis at birth that leads to anaemia, pallor or jaundice.

Haemolysis usually disappears in the first three years of life.

Adults are asymptomatic or have only minimal haemolytic anemia.

SAO results from a 27 bp deletion in the SLC4A1 gene, localized on chromosome 17q21.31 (SLC4A1del27 mutation).

Heterozygous mutations for the deletion are found in almost all cases.

Homozygosity is thought to be lethal to the developing embryo.
High prevalence of Southeast Asian ovalocytosis in Malays with distal renal tubular acidosis
Noraziah Mohd Yusoff, Hans Van Roodenbergh
Published 2000 in Journal of Human Genetics
DOI: 10.1007/s12046-005-0295-2

Abstract: Southeast Asian ovalocytosis (SAO) is a red blood cell abnormality common in malaria-endemic regions and caused by a 27 nt deletion of the band 3 protein gene. Since band 3 protein, also known as anion exchanger 1, is expressed in renal distal tubules, the incidence of SAO was examined in distal renal tubular acidosis (dRTA) in Malaysia. In Kelantan, Malaysia, twenty-two patients with dRTA and 50 healthy volunteers were examined for complication of SAO by both morphological and genetic analyses. SAO was identified in 18 of the 22 dRTA patients (81.8%), but only two of the 50 controls (4%). The incidence of SAO was significantly high in those with dRTA (p<0.001), indicating a dysfunctional role for band 3 protein/anion exchanger 1 in the development of dRTA.

Fig. 1. A morphological finding of Southeast Asian ovalocytosis (SAO): Ovalocytic changes are observed in more than 23% of red blood cells in peripheral blood smear. Arrow indicates a representative ovalocyte. b) PCR amplification products of the band 3 protein/anion exchanger 1 gene: Two amplified products corresponding to 175 bp and 148 bp were obtained from all samples of patients diagnosed with SAO (P) but only one product was found in normal controls (N). M, P and N refer to a size maker, patient and normal, respectively.
Homozygous Southeast Asian ovalocytosis is a severe dyserythropoietic anemia associated with distal renal tubular acidosis

Véronique Picard, Alexis Proust, Marion Eveillard, Joanna F. Flatt, Marie-Laure Couec, Gaëlle Caillaux, Madeleine Fénéant-Thibault, Arie Finkelstein, Martine Raphaël, Jean Delaunay, Lesley J. Bruce, Serge Pissard, and Caroline Thomas

CO-INHERITANCE OF SOUTHEAST ASIAN OVALOCYTOSIS WITH B-THALASSEMIA OR HEMOGLOBIN E HETEROZYGOTE LEAD TO ANEMIA IN CHILDREN

Author(s): Pallapa Banjerdlak, cronun sikomsawan, thirachit chotsampancharoen, malai wonchanchailed, Natsarut Songthawoo, Sarapoju Duanchøy
EHA Learning Center. banjerdlak p. Jun 15, 2016, 215201

However, co-inheritance of SAO with β-thalassemia heterozygote has rarely been reported. It is still not known how SAO and β-thalassemia interact, nor its effect on clinical phenotype.

Aims
To determine the effects of co-inheritance of SAO with β-thalassemia, or Hb E heterozygote on clinical severity, hematological parameters compared to SAO patients without these mutations.

Conclusion
Co-inheritance of SAO coupled with β-thalassemia did not affect the degree of neonatal anemia and jaundice at birth, however it lead to a higher incidence of anemia when patients reached one year of age. The presence of β-thalassemia mutation within the SAO population significantly increased the degree of anisocytosis.

Target cells
The term **knizocyte** describes a specific shape form of erythrocytes that deviates from the standard form. These cells have two concavities instead of the one seen with normal erythrocytes. Like normal red blood cells, knizocytes show a clear central area, but this is crossed by a thin strip of hemoglobin. It can be seen particularly in patients with thalassemia.
Diagnostic methods

• Diagnosis is based on the presence on a peripheral blood smear of macro-ovalocytes, some of them stomatocytic with more than one stoma.

• Genetic assays can also be used to identify the mutation in the SLC4A1 gene.

“The diagnosis of SAO is made based on peripheral blood smears that had a presence of more than 25% of elliptocytes, macro-ovalocytes and stomatocytes (so called Theta cells “θ”).

PCR based - molecular analysis to confirm patients are heterozygous 27bp-deletion of AE1 gene.”
Accurate light microscopic diagnosis of South-East Asian ovalocytosis

INTRODUCTION: South-East Asian ovalocytosis (SAO) is a common inherited red blood cell polymorphism in South-East Asian and Melanesian populations, coinciding with areas of malaria endemicity. Validation of light microscopy as a diagnostic alternative to molecular genotyping may allow for its cost-effective use either prospectively or retrospectively by analysis of archived blood smears.

METHODS: We assessed light microscopic diagnosis of SAO compared to standard PCR genotyping. Three trained microscopists each assessed the same 971 Giemsa-stained thin blood films for which SAO genotypic confirmation was available by PCR. Generalized mixed modeling was used to estimate the sensitivity, specificity, positive predictive value, and negative predictive value of light microscopy vs “gold standard” PCR.

RESULTS: Among red cell morphologic parameters evaluated, knizocytes, rather than ovalcytic morphology, proved the strongest predictor of SAO status (odds ratio [OR] = 19.2; 95% confidence interval [95% CI] = 14.6-25.3; P ≤ 0.0001). The diagnostic performance of a knizocyte-centric microscopic approach was microscopist-dependent: two microscopists applied this approach with a sensitivity of 0.63 and a specificity of 0.93. Inter-rater reliability among the microscopists (κ = 0.20) as well as between gold standard and microscopist (κ = 0.36) underperformed due to misclassification of stomatocytes as knizocytes by one microscopist, but improved substantially when excluding the error-prone reader (κ = 0.65 and 0.74, respectively).

CONCLUSION: Light microscopic diagnosis of SAO by knizocyte visual cue performed comparable to time-consuming and costlier molecular methods, but requires specific training that includes successful differentiation of knizocytes from stomatocytes.

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REQUEST FORM – GO005/19
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<th>Reference Ranges</th>
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<td>Dr. Nazrul Mohd Yusoff</td>
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**Advanced Diagnostic Laboratory**

**Kompleks KLINIKAL (Clinical Complex)**

**Institut Perubatan dan Perigian Termaju (IPPT)**

**Universiti Sains Malaysia**

13290, Kepala Bistri, Pulau Pinang, Tel: 04-6237711

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**Diagnosis:** SAD

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**Differential Count**

- Neutrophils %: L 32.0 %, 43.2 - 70.5
- Lymphocytes %: H 58.0 %, 19.02 - 47.54
- Monocytes %: 4.0 %
- Eosinophil %: 0.0 %
- Basophil %: 0.0 %

**Peripheral Blood Film**

- Method: Wright’s stain
- Hb:
- RBC: Normal red blood cell count. Slight anisocytosis and hypochromic microcytic red cells, oval stomatocytes, target cells seen.

Verified by: Prof. Dr. Norawati Mohd Yusoff
<table>
<thead>
<tr>
<th>TEST</th>
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<tr>
<td>Platelets</td>
<td>Normal platelet count and morphology.</td>
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<td>Impression</td>
<td>1. Red blood cell changes - Southeast Asian Ovalocytosis (SAO) with concurrent Thalassemia. 2. White blood cell changes suggestive of inflammation/infection.</td>
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<td>Suggestion</td>
<td>For molecular study for confirmation of SAO.</td>
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RESULTS

**Electrophoresis**
2% Agarose Gel (90w and 70 min).

**Indicator:**
- L1: Ladder 100bp
- L2: Negative control
- L3: Normal control
- L4: Positive control
- L5: GO 005/19
- L6: GO 006/19

PCR analysis shows heterozygosity for the SFO Band 3 deletion.

29/6/2019
**RESULTS**

**ADVANCED DIAGNOSTIC LABORATORY**
**INSTITUT PERBUNATAN DAN PENGIGIAN TERMASU (IPPT)**
**UNIVERSITI SAINS MALAYSIA**

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**MOLECULAR GENETICS**

**DNA Analysis - Southeast Asian Ovocystis**

- **Section Lab No.**: GO00519
- **Specimen**: Blood
- **Method**: DNA Extraction, PCR SAC
- **Result**: PCR analysis shows heterozygosity for the Southeast Asian ovocystis (SAC) Band 3 variation.

*Please send in parental blood for further investigation.*

- **Reported by**: Recedtal Ali, Razali / Prof. Dr. Narazah
- **Date**: 25/06/19

Verified by: Prof. Dr. Narazah Mohd Yasrif

**PROF.DR. NARAZAH MOHD YASrif**
**NO. RM- 36573**
**Fakulti Perubatan Kedokteran / Genetika**
**Instiit Universiti Sains Malaysia**
Common features of RBC membrane disorders

**Features**
- loss of surface area
- change in morphology

**Outcome**
- Tendency to splenic sequestration and extravascular haemolysis
- chronic anaemia of variable severity
LABORATORY DIAGNOSIS OF RED CELL MEMBRANE DEFECTS

Clinical and family history
Red cell indices
Peripheral blood smear examination
Results from relevant laboratory tests for indicating a haemolytic process and a membrane defect
## Diagnostic Performance of Screening Tests for Hereditary Spherocytosis

**Table 1. Application of screening tests in the differential diagnosis of hereditary spherocytosis and other membrane-associated red cell disorders**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Osmotic fragility test</th>
<th>Acid glycerol lysis time test</th>
<th>EMA-binding test</th>
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<tbody>
<tr>
<td>Hereditary spherocytosis</td>
<td>↑ fragility</td>
<td>Shortened lysis time</td>
<td>↓ fluorescence</td>
</tr>
<tr>
<td>Auto-immune hemolytic anemia</td>
<td>↑ fragility</td>
<td>Shortened lysis time</td>
<td>Normal or ↑ with some cases ↓↓ fluorescence</td>
</tr>
<tr>
<td>Hereditary pyropoikilocytosis</td>
<td>?</td>
<td>?</td>
<td>↑ fluorescence</td>
</tr>
<tr>
<td>Overhydrated hereditary stomatocytosis</td>
<td>↑ fragility</td>
<td>?</td>
<td>Normal or ↑ fluorescence</td>
</tr>
<tr>
<td>Dehydrated hereditary stomatocytosis</td>
<td>↓ fragility</td>
<td>Normal lysis time</td>
<td>Normal or ↓ fluorescence</td>
</tr>
<tr>
<td>Cryohydrocystosis</td>
<td>?</td>
<td>?</td>
<td>↓ fluorescence</td>
</tr>
<tr>
<td>Congenital dyserythropoietic anemia type II</td>
<td>↑ fragility</td>
<td>Shortened lysis time with some cases</td>
<td>Normal or ↓ Fluorescence with some cases ↓ fluorescence</td>
</tr>
<tr>
<td>Southeast Asian ovalocytosis</td>
<td>?</td>
<td>?</td>
<td>↓ fluorescence</td>
</tr>
</tbody>
</table>

?: No published data found.

M.-J. King and A. Zanella | Laboratory Diagnosis of Membranopathies 5s
Differential diagnosis can often be misdiagnosed with other hemolytic anemias. HS can be confused with autoimmune hemolytic anemia that shows spherocytes on the PB smear. Perform additional diagnostic tests - Coombs’ test. Other conditions: spherocytes include liver disease, thermal injury, micro- and macroangiopathic hemolytic anemias, clostridial sepsis, transfusion reaction with hemolysis, severe hypophosphatemia poisoning with certain snake, spider. It is critical to evaluate the disorder in the proper clinical context and to evaluate the family history and transmission pattern.
Differential diagnosis

Enzymatic defects, G6PD) and pyruvate kinase (PK deficiencies

If suspected, the diagnosis should be confirmed by performing the most common red cell enzyme assays

Analyse the inheritance of the disease that is X-linked for G6PD deficiency and autosomal recessive for PK defect.
Flow diagram for the differential diagnosis of hemolytic anemias due to RBC membrane defects.
Challenges in diagnosis

- Heterogeneous with overlapping phenotypes
- Unlike other diseases – can have biomarkers (genetic mutations, based on morphology),
- May overlap with other conditions (morphology etc),
- Incidental finding usually but can have severe manifestations
- Diagnosis of typical HS is straightforward, the current difficulty lies with making a firm diagnosis of HS for those patients presenting with an intermittent hemolysis and occasional spherocytosis
- Concomitant causes of haemolysis should been considered and ruled out in difficult cases (enzyme or haemoglobin defects)
- The absence of clear genotype/phenotype correlations is often problematic for both genetic counseling and suitable treatments.
Challenges in diagnosis

Some subtypes of RBC membrane disorders can be easily confused with other clinically-related hereditary hemolytic conditions, as classically reported for differential diagnosis of HS and CDA II.

Thus, when suspected, after the exclusion of other common diseases, it is essential to perform a depth analysis of PB smear and pedigree transmission of the disease.

Biochemical tests can be useful, especially in HS, but they do not have high sensitivity.

The combination of an EMA test with ektacytometry is of great help for the majority of these conditions, but ektacytometry analysis is of limited availability.

Thus, the genetic analysis becomes crucial, mainly in cases with an ambiguous phenotype.
Next Generation Sequencing (NGS)

Genetic testing - test for few candidate genes to wider panels of genes, namely targeted (t)-NGS.

Recent studies - usefulness of t-NGS as a comprehensive and invaluable diagnostic tool:
• achieving a correct diagnosis
• proceeding with careful management of these patients
t-NGS

Accelerate the analysis, reduce costs and provide a clear diagnosis

Ability to be easily upgradable in view of novel discoveries

Major drawback of current NGS applications - is represented by the data processing steps

Difficulty in determining the pathogenicity of the numerous identified variants.

Overcome this limitation - the simultaneous evaluation of all family members

Establish the inheritance pattern of the identified variants to understand its pathogenetic role
Hereditary anaemias due to RBC membrane defects represent a heterogeneous group of hereditary defects with very overlapping phenotypes.

Indeed, the clinical definition of patients is often difficult.

For some conditions, the great phenotypic variability is partially explained by the high genetic heterogeneity.

Otherwise, it is sometimes complicated to distinguish one form from the others since the signs can be veiled in symptom-free carriers or in mildly affected patients.
Conclusion

It is proper in this context that new genomic technologies are utilized.

In the last few years, remarkable progress has been made in discovering new disease genes involved in these disorders by means of unbiased genomic approaches, e.g. exome sequencing.

However, the increasing genetic heterogeneity underlines the problem of a very complex differential diagnosis.

Recent studies - usefulness of t-NGS as a comprehensive and invaluable diagnostic tool.
References


• Hereditary red cell membrane disorders and laboratory diagnostic testing M.-J. KING*, A. ZANELLA†© 2013 Blackwell Publishing Ltd, Int. Jnl. Lab. Hem


• Anatomy of the red cell membrane skeleton: unanswered questions, Samuel E. Lux IV Blood 2016 127:187-199; doi: https://doi.org/10.1182/blood-2014-12-512772


THANK YOU